

CLAIMS

1. A method for identifying an interacting target biomolecule to a biomolecule of interest comprising the steps of:
 - (a) providing a biomolecule of interest having specificity for the target;
 - (b) binding the biomolecule of interest to at least one type of linker molecule, the linker molecule comprising at least one attachment part for binding to the biomolecule of interest, one cleavable part, one mass marker part and one photoactivatable part, for binding to the target;
 - (c) contacting the biomolecule of interest with a cell or a cell extract;
 - (d) exposing the cell to photolysis, whereby the photoactivatable part binds to the target;
 - (e) cleaving the linker molecule, thereby leaving the photoactivatable part and the mass marker part bound to the target;
 - (f) analysing the product of step (e), thereby detecting the mass marker part, thus identifying the interacting target biomolecule to the biomolecule of interest.
2. A method according to claim 1, wherein the biomolecule of interest is a protein or a peptide.
3. A method according to claim 1, wherein the target biomolecule is a protein or a peptide.
4. A method according to any one of the preceding claims, wherein the affinity of the biomolecule of interest for the target biomolecule is in the interval of 10 mM to 0.1 pM.
5. A method according to any one of the preceding claims, wherein the attachment part of the linker molecule is designed to bind to a specific amino acid residue of the biomolecule of interest.
6. A method according to any one of the preceding claims, wherein the attachment part of the linker molecule is a N-hydroxysuccinimide moiety or a N-maleimide.
7. A method according to any one of the preceding claims, wherein the cleavable part of the linker molecule is cleaved by chemical means.

8. A method according to claim 6, wherein the cleavable part is cleaved by an oxidising agent or by a base agent.
9. A method according to any one of the preceding claims, wherein the cleavable part is a geminal diol or an ester linkage.
10. A method according to any one of the preceding claims, wherein the mass marker part has the ability to fragment during the analysis step.
11. A method according to any one of the preceding claims, wherein the mass marker part is thioethylpyridine.
12. A method according to any one of the preceding claims, wherein at least two different linker molecules are used.
13. A method according to any one of the preceding claims, wherein the photoactivatable part is an azide or a benzophenone.
14. A method according to any one of the preceding claims, wherein the linker molecule is 2-benzophenon-4-yl-carbonylamino-4, 5-dihydroxy-6-(N-succinimidyl)-1-(4-pyridylethylthio)-3-n-hexanone.
15. A method according to any one of the preceding claims, wherein the linker molecule further comprises a fluorescent protein tag.
16. A method according to any one of the preceding claims, wherein the linker molecule comprises a tag directing it to a subcellular location.
17. A method according to any one of the preceding claims, wherein the cell of step (c) is perforated, in the form of a cell extract or in a cell-free translation system.
18. A method according to any one of the preceding claims, wherein the photolysis of step (d) is performed by exposing the cell to UV-light.
19. A method according to step 18, wherein the photolysis is repeated at least once.
20. A method according to any one of the preceding claims, wherein the product of step (d) is chemically and/or enzymatically digested.
21. A method according to claim 20, wherein the digestion is performed by cyanogen bromide and/or trypsin.
22. A method according to any one of the preceding claims, wherein step (f) is multidimensional HPLC coupled to a mass spectrometer (MS).

23. A method according to claim 22, wherein the MS is in a parent ion scanning mode.
24. A method according to claim 23, wherein the molecules comprising the marker is detected at 106 m/z.
25. A method according to claim 24, wherein the MS operates in a data-dependent mode, thereby switching from parent ion to daughter ion scanning mode when a peptide containing the marker is detected.
26. Use of the linker molecule 2-benzophenon-4-yl-carboxylamino-4, 5-dihydroxy-6-(N-succinimidyl)-1-(4-pyridylethylthio)-3-n-hexanone for labelling a specific target biomolecule for MS parent ion scanning.
27. A kit for use in the method according to claim 1-26 comprising at least one linker molecule, optionally together with a biomolecule of interest in separate compartments.